Remarks

Applicant respectfully requests consideration of the remarks submitted herein. Claims 1-28, 30-31, 33, 40-42, and 47-71 are pending.

Rejection under 35 U.S.C. § 103(a)

Claims 1-28, 30-31, 33, 40-42, and 47-71 were rejected under 35 U.S.C. 103(a) as unpatentable over WO 99/13816 in combination with Tardi (US 2003/0124181). This rejection is respectfully traversed.

WO 99/13816 discusses a method for loading liposomes with camptothecins. At page 2 of the Office action, the Eaxminer notes that what is lacking in WO is the loading of active agents other than camptothecins.

At page 3 of the Office action, the Examiner states that Tardi teaches that therapeutic agents that comprise one or more ionizable moiety can be loaded using pH gradients. It is noted that Tardi primarily focuses on the preparation of liposomes from negatively charged lipids that are stable in the blood (please see Tardi at the Abstract, and at paragraphs 0013-0020). At paragraphs 0072-0083, Tardi does generally discuss both the passive and active loading of liposomes. However, gradient loading is not the focal point of the Tardi invention.

Independent claims 1, 63, and 71 recite the following:

> (a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;
>
> (b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;
>
> (c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes;
>
> (d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

The Examiner has taken the position that it would have been obvious from the teaching of Tardi to load the anthracyclic antibiotics and vinca alkaloids recited in the instant claims using the method discussed in WO 99/13816.

The Supreme Court set out the analysis for patentability under 35 USC 103(a): the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background the obviousness or nonobviousness of the subject matter is determined (*see, e.g., Graham v. John Deere Co.*, 383 U.S. 1 (1966) and *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (2007)).

The instant claims include a quenching step (d), which recites contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes. As discussed at page 14 of the specification,

> Drug loading via the pH gradient includes a low pH in the internal aqueous space of the liposomes, and this internal acidity is, by design, incompletely neutralized during the drug loading process. This residual internal acidity can cause chemical instability in the liposomal preparation (e.g., lipid hydrolysis), leading to limitations in shelf life. To quench this residual internal acidity, membrane permeable bases, such as amines (e.g., ammonium salts or alkyl-amines) can be added following the loading of the pharmaceutical agent in an amount sufficient to reduce the residual internal acidity to a minimum value (for example, pH at or above 4).

This quenching step represents a significant difference between the teaching of Tardi and the instant claims.

Tardi does not teach the preparation of any liposomes using the quenching step (d). Accordingly, the final liposomes prepared by Tardi maintain a low pH in the internal aqueous space that helps keep the drug loaded inside the liposome. At paragraph 0076 Tardi teaches that "Once the drug moves inside the liposome, the pH of the interior results in a charged drug state, which prevents the drug from permeating the lipid bilayer, thereby entrapping the drug in

the liposome." Additionally in paragraph 0080, Tardi teaches that "Conversion of moiety to a charged form causes the drug to remain encapsulated within the liposome." Accordingly, Tardi teaches that it is critical to maintain the low pH in the internal aqueous space after active loading of the liposome in order to keep the therapeutic agent trapped inside.

Applicant respectfully submits that one skilled in the art would not have had a reasonable belief that the anthracycline antibiotics and the vinca alkaloids generally discussed in Tardi could have been loaded using the method of WO 99/13816 to provide a loaded liposome that would retain the anthracycline antibiotic or the vinca alkaloid following the quenching procedure of WO 99/13816, since Tardi teaches that the low pH in the interior of the liposome is critical for keeping the agent inside the liposome following gradient loading. Thus, Tardi teaches away from employing the quenching step (d) recited in the instant claims. Accordingly, it is respectfully submitted that one skilled in the art would not have been motivated to combine the teaching of WO 99/13816 and Tardi as suggested by the Examiner. It is also respectfully submitted that one skilled in the art would not have had a reasonable expectation that the liposomes discussed by Tardi would have effectively retained the anthracycline antibiotic or the vinca alkaloid if the residual internal acidity was quenched following loading using the method of WO 99/13816.

Additionally, at page 2, section 2 of the Offfice action, the Examiner states that "Although in Examples, WO uses citric acid at 50 mM concentration, in view of WO's teachings that it can be higher than 5mM, it would have been obvious to one of ordinary skill in the art to vary the molality with the expectation of obtaining the best possible results." For the following reasons, Applicant respectfully disagrees with the Examiner's statement.

Gradient loading using a lower concentration of citric acid produces a final liposome with a small amount of residual acid present, since most of the internal acid is consumed by the therapeutic agent within the liposome. Accordingly a liposome loaded at a lower concentration of citric acid (e.g. 50 mM) would only require a small amount of base to quench the residual acid present after loading. When a higher concentration of citric acid (e.g., greater than 60 mM) is utilized during a gradient loading process, the amount of therapeutic agent present in the final liposomes is not great enough to neutralize a majority of the residual acid. Accordingly when liposomes are loaded at a higher acid concentration there is a significantly greater amount of residual acid remaining in the liposomes following loading. Accordingly, it is submitted one

skilled in the art would not have had a reasonable expectation that liposomes could be loaded at a higher concentration of acid (e.g., greater than 60 mM) and subsequently quenched as recited in the instant claims after viewing the examples in WO 99/13816, since one skilled in the art would have appreciated that the amount of residual acid in a liposome loaded at a higher concentration of citric acid (e.g., greater than 60 mM) would have been significantly higher than the amount of residual citric acid present in a liposome loaded at a concentration of 50 mM as discussed in the Examples of WO 99/13816.

Additionally, one skilled in the art would have believed that gradient loading using a high concentration of citric acid would produce a final liposome with a higher concentration of therapeutic agent in the liposome than would be produced by gradient loading at a lower concentration of citric acid. Accordingly one skilled in the art would have known that gradient loading using a higher concentration of acid would produce a final liposome that is potentially less thermodynamically stable, i.e. a liposome wherein the therapeutic agent is more likely to leak or escape from the liposome. As discussed above, Tardi teaches that it is critical to maintain low pH in the internal aqueous space after active loading of the liposome in order to keep the therapeutic agent trapped inside. One skilled in the art would have understood that the teaching of Tardi would have been more relevant for liposomes loaded at higher gradients, since these liposomes contain more therapeutic agent and thus, are more likely to leak if the pH gradient is quenched. For this additional reason, it is submitted that it would not have been obvious to one of ordinary skill in the art to vary the molality (by increasing the concentration of citric acid) with the expectation of obtaining the best possible results, as suggested by the Examiner. In fact, it is submitted that one skilled in the art would have more likely assumed that quenching liposomes that were gradient loaded at a high concentration of citric acid would have produced liposomes that were unstable (i.e. liposomes where the therapeutic agent would leak from the liposome) in light of the discussion in Tardi.

Since there was no motivation to combine the cited documents as suggested by the Examiner, and because there would not have been a reasonable expectation that the references so combined would have provided liposomes that would have sufficiently retained the therapeutic agent, it is respectfully submitted that the Examiner has not established a *prima facie* case of obviousness over the disclosure of WO 99/13816 in combination with Tardi. Accordingly, withdrawal of the rejection is appropriate and is requested.

It is noted that the inclusion of claim 7 (which recites sphingomyelin) in this rejection is believed to have been an error. At page 3 and again at page 4 of the Office action, the Examiner admitted that these cited documents do not teach sphingomyelin as a liposome-forming lipid. Accordingly, the cited documents do not teach all elements of claim 7. Thus, claim 7 can not be *prima facie* obvious over the cited documents. Additionally, at page 3 of the Office action the Examiner states that "since it is a commonly used lipid in the liposome formations, it would have been obvious to one of ordinary skill in the art to use this lipid with a reasonable expectation of success." It is respectfully submitted that the Examiner has provided no evidence to support this conclusion. Since the Examiner provided no evidence to support this conclusion, Applicant submits that the Examiner is taking "official notice" that "it would have been obvious to one of ordinary skill in the art to use this lipid with a reasonable expectation of success." If the Office maintains the rejection of claim 7 over WO 99/13816 in combination with Tardi, under 37 C.F.R. 1.104(d)(2), the Examiner must provide an affidavit or declaration setting forth specific factual statements and explanation to support this finding. Thus, if the Office maintains the rejection, in the next communication Applicant respectfully requests that the Examiner provide an affidavit or declaration setting forth specific factual statements to support the conclusion that it would have been obvious to one of ordinary skill in the art to use this lipid with a reasonable expectation of success.

It is also noted that the inclusion of claim 49 in this rejection is believed to have been an error. At page 4 of the Office action, the Examiner admitted that these cited documents do not teach the change of the pH using methylamine. Accordingly, the cited documents do not teach all elements of claim 49. Thus, claim 49 can not be *prima facie* obvious over the cited documents.

It is also noted that the inclusion of claims 52-57 in this rejection is believed to have been an error. The Examiner has not suggested that the cited documents teach dehydration of liposomes in the presence of cryoprotectants (also, please see section 5 of the Office action). Accordingly, the cited documents do not teach all elements of claims 52-57. Thus, claims 52-57 can not be *prima facie* obvious over the cited documents.

In light of the above remarks, it is respectfully requested that the rejection of claims 1-28, 30-31, 33, 40-42, and 47-71 under 35 U.S.C. 103(a) as unpatentable over WO 99/13816 in combination with Tardi (US 2003/0124181) be withdrawn.

Claims 1-28, 30-31, 33, 40-42, and 47-71 were rejected under 35 U.S.C. 103(a) as unpatentable over EP 0 719 546. This rejection is respectfully traversed.

EP 0 719 546 discusses gradient loading of liposomes – however, EP 0 719 546 does not disclose a process that includes steps (c) and (d) above. EP 0 719 546 also notes that none of the previous liposomal formulations of doxorubicin fully satisfy fundamental stability requirements, since doxorubicin is not typically maintained for a long enough period of time in the liposomes (see column 4 therein). EP 0 719 546 discusses liposomal formulations that use a transmembrane pH gradient to improve the stability of liposomal formulations of antineoplastic agents (column 6 therein). Accordingly, EP 0 719 546 teaches that a transmembrane pH gradient can be used to reduce the rate of unwanted drug release from liposomal formulations (column 8 therein).

EP 719 546 does not disclose a liposome forming method that includes step (c) and step (d) that are recited in the instant claims. Accordingly, EP 719 546 does not teach all the elements of the instant claims. Thus, the instant claims are not *prima facie* obvious over the disclosure of EP 719 546. Accordingly, withdrawal of the rejection of claims 1-23, 30, 40-42 and 47-71 under 35 U.S.C. 103(a) as unpatentable over EP 0 719 546 is appropriate and is requested.

At page 4, lines 5-7 of the Office action, the Examiner concluded that "it would have been obvious to one of ordinary skill in the art to load an active agent at an acidic medium and then relative to the liposome interior and then change the pH of the exterior to basic pH such that the active agent remains entrapped." (Office action at page 4 lines 5-7). As discussed above, EP 0 719 546 does not suggest the preparation of any liposomes using steps (c) and (d) recited in the instant claims. Additionally, EP 0 719 546 discusses a transmembrane pH gradient that is used to improve the stability of liposomal formulations of antineoplastic agents (column 6 therein); and EP 0 719 546 teaches that a transmembrane pH gradient can be used to reduce the rate of unwanted drug release from liposomal formulations (column 8 therein). Thus, EP 0 719 546 teaches that a transmembrane pH gradient is necessary to provide suitable drug retention in the liposomes therein. Accordingly, it is respectfully submitted that EP 0 719 546 teaches away from quenching the pH gradient after loading – since EP 0 719 546 intentionally establishes this gradient and teaches that it is critical for preventing unwanted drug release from the liposomes. Thus, one skilled in the art would not have reasonably concluded from the teaching of EP 0 719

546 that the anthracycline chemotherapeutic agent, anthracenedione, or the vinca alkaloid recited in the instant claims would remain in the liposomes if the internal acidity was quenched after loading. In fact, EP 0 719 546 teaches exactly the opposite. Thus, it is submitted that one skilled in the art would not have been motivated to subject the liposomes of EP 0 719 546 to step (d) as recited in the instant claims, since there would not have been a reasonable expectation that the liposomes would have retained the loaded drug following treatment under the conditions of step (d). Accordingly, it is respectfully submitted that conclusion of the Examiner at page 4, lines 5-7 of the Office action is incorrect.

Finally, the Examiner has not provided any evidence to support his conclusion that "it would have been obvious to one of ordinary skill in the art to load an active agent at an acidic medium and then relative to the liposome interior and then change the pH of the exterior to basic pH such that the active agent remains entrapped." Since there is no evidence of record to support this conclusion, Applicant submits that the Examiner is taking "official notice" that "it would have been obvious to one of ordinary skill in the art to load an active agent at an acidic medium and then relative to the liposome interior and then change the pH of the exterior to basic pH such that the active agent remains entrapped." If the Office maintains the rejection over EP 0 719 546, under 37 C.F.R. 1.104(d)(2), the Examiner must provide an affidavit or declaration setting forth specific factual statements and explanation to support this finding. Thus, if the Office maintains the rejection, in the next communication Applicant respectfully requests that the Examiner provide an affidavit or declaration setting forth specific factual statements and explanation to support the conclusion that it would have been obvious to one of ordinary skill in the art to load an active agent at an acidic medium and then relative to the liposome interior and then change the pH of the exterior to basic pH such that the active agent remains entrapped.

At page 4 of the Office action, regarding phosphatidylglycerol, the Examiner states that "since it is the commonly used negatively charged lipid to provide negative charge to the liposomes, it would have been obvious to one of ordinary skill in the art to include this phospholipid with a reasonable expectation of success." It is respectfully submitted that the Examiner has provided no evidence to support this conclusion. Why would one skilled in the art have even considered phosphatidylglycerol; why would one skilled in the art have wanted to use it; what effect would one skilled in the art have assumed it would have had in the particular systems recited in the claims? Since the Examiner provided no evidence to support

this conclusion, Applicant submits that the Examiner is taking "official notice" that "it would have been obvious to one of ordinary skill in the art to use this lipid with a reasonable expectation of success." If the Office maintains the rejection, under 37 C.F.R. 1.104(d)(2), the Examiner must provide an affidavit or declaration setting forth specific factual statements and explanation to support this finding. Thus, if the Office maintains the rejection, in the next communication Applicant respectfully requests that the Examiner provide an affidavit or declaration setting forth specific factual statements to support the conclusion that it would have been obvious to one of ordinary skill in the art to include this phospholipid with a reasonable expectation of success.

It is noted that the inclusion of claim 7 in this rejection is believed to have been an error. At page 4 of the Office action, the Examiner admitted that EP 0 719 546 does not teach sphingomyelin as a liposome-forming lipid. Accordingly, the cited document does not teach all the elements of claim 7. Thus, claim 7 can not be *prima facie* obvious over the cited documents.

It is also noted that the inclusion of claim 49 in this rejection is believed to have been an error. At page 4 of the Office action, the Examiner admitted that these cited documents do not teach the change of the pH using methylamine. Accordingly, the cited documents do not teach all elements of claim 49. Thus, claim 49 can not be *prima facie* obvious over the cited documents.

It is also noted that the inclusion of claims 52-57 in this rejection is believed to have been an error. The Examiner has not suggested that the cited documents teach dehydration of liposomes in the presence of cryoprotectants (also, please see section 5 of the Office action). Accordingly, the cited documents do not teach all elements of claims 52-57. Thus, claims 52-57 can not be *prima facie* obvious over the cited documents.

Claims 7 and 49 were also rejected under 35 USC § 103(a) as being as being unpatentable over WO 99/13816 in combination with Tardi OR over EP 0 719 546 as set forth above, further in view of Webb (5,814,335).

At page 4 of the Office action the Examiner stated that what is lacking in WO 99/13816, Tardi, and EP 0 719 546 is the use of sphingomyelin as a liposome forming lipid. As discussed above, independent claims 1, 63, and 71 are not *prima facie* obvious over WO 99/13816, Tardi, and EP 0 719 546 as applied earlier in the Office action. Thus, more is lacking from the primary

documents than the use of sphingomyelin. It is respectfully submitted that the secondary document Webb, as applied by the Examiner, does not cure the deficiencies discussed above, since it was only cited with respect to sphingomyelin as a liposome forming lipid. Accordingly, claims 7 and 49 are not obvious over the disclosures of WO 99/13816 in combination with Tardi or over EP 0 719 546 as set forth above, further in view of Webb (5,814,335). Withdrawal of this rejection is respectfully requested. It is not clear why claim 49 was included in this rejection relating to sphingomyelin. Clarification is requested.

Additionally at page 4, the Examiner states that neither EP nor WO teaches "the change of the pH of the external medium by using methylamine," and that "The use of methylamine to change the pH of the external medium would have been obvious to one of ordinary skill in the art with a reasonable expectation of success since Webb teaches the creation of a pH gradient using methylamine (columns 7 and 8)." As discussed above, independent claims 1, 63, and 71 are not *prima facie* obvious over WO 99/13816, Tardi, and EP 0 719 546 as applied earlier in the Office action. Thus, more is lacking from the primary documents than the use of methylamine as a base. It is respectfully submitted that the secondary document Webb, as applied by the Examiner, does not cure the deficiencies, since it was only cited with respect to the use of methylamine. Accordingly, it is submitted that claims 7 and 49 are not obvious over the disclosures of WO 99/13816 in combination with Tardi or over EP 0 719 546 as set forth above, further in view of Webb (5,814,335). Withdrawal of this rejection is respectfully requested.

In addition to the fact that the rejection should be withdrawn for the reason discussed above, it is respectfully submitted that methyl amine/methyl ammonium gradient discussed in Webb performs a significantly different function that the methyl amine recited in claim 49. Webb discusses the use of a methyl amine/methyl ammonium gradient to actively load the neutral form of a protonatable therapeutic agent (column 7, lines 40-64) into a liposome. Claim 49 is directed to the method of claim 1 wherein a weak base selected from the group of methyl amine, ethyl amine, diethyl amine, ethylene diamine, and propyl amine is used in step (d) to quench the residual acidity inside the liposomes after loading in the presence of an acid.

Webb does not use the methylamine to quench residual acid in a loaded liposome. Rather, Webb uses methylamine to establish a pH gradient (as noted by the Examiner at page 5 of the Office action). Thus, methyl amine is used by Webb for the opposite purpose than it is used for in the claimed methods. Accordingly, it is submitted that one skilled in the art would

not have found any motivation in Webb to use methylamine as recited in claim 49. For this additional reason it is submitted that claims 7 and 49 are not obvious over the disclosures of WO 99/13816 in combination with Tardi or over EP 0 719 546 as set forth above, further in view of Webb (5,814,335) as suggested by the Examiner. Withdrawal of this rejection is respectfully requested. It is not clear why claim 7 was included in this rejection relating to methylamine. Clarification is requested.

Claims 52-57 were rejected under 35 USC § 103(a) as unpatentable over WO 99/13816 in combination with Tardi OR over EP 0 719 546 as set forth above, further in view of Clerc (5,939,096).

At page 5 of the Office action the Examiner stated that Clerc teaches liposomes can be dehydrated for storage in the presence of cryoprotectants. As discussed above, independent claims 1, 63, and 71 are not *prima facie* obvious over WO 99/13816, Tardi, and EP 0 719 546 as applied earlier in the Office action. Thus, more is lacking from the primary documents than the dehydration of liposomes in the presence of cryoprotectants. It is respectfully submitted that the secondary document Clerc, as applied by the Examiner, does not cure the deficiencies discussed above, since it was only cited with respect to the dehydration of liposomes in the presence of cryoprotectants. Accordingly, the claims 52-57 are not obvious over the disclosures of WO 99/13816 in combination with Tardi or over EP 0 719 546 as set forth above, further in view of Clerc (5,939,096). Withdrawal of this rejection is respectfully requested.

Claims 1-28, 30-31, 33, 40-42, and 47-71 were provisionally rejected on the ground of non-statutory obviousness-type double patenting over claims 1-23, 30, 40-42, and 47-71 of co-pending Application No. 10/723,610.

Independent claims 1, 63, and 71 of co-pending application 10/723,610 have been amended to recite "contacting liposomes in an aqueous solution of up to 50 mM" in step (a). This amendment is believed to obviate the Examer's ground for rejection. Accordingly, withdrawal of this rejection is respectfully requested.

If the Examiner maintains this rejection, Applicant will wait until otherwise patentable subject matter is identified in both cases before determining if a terminal disclaimer is appropriate, since this is a provisional rejection.

Claims 1-28, 30-31, 33, 40-42, and 47-71 were rejected on the ground of non-statutory obviousness-type double patenting over claims 30-31 and 35-64 of U.S. Patent Number 6,740,335 in combination with Tardi. This rejection is respectfully traversed.

WO 99/13816 (discussed above) is a foreign counterpart for U.S. Patent 6,740,335. Accordingly, it is submitted that the instant claims are not obvious over the claims 30-31 and 35-64 of U.S. Patent Number 6,740,335 in combination with Tardi for the reasons detailed above in the Remarks relating to the rejection over WO 99/13816 in combination with Tardi (US 2003/0124181). Accordingly, withdrawal of this rejection is respectfully requested.

If the Examiner maintains this rejection, Applicant will wait until otherwise patentable subject matter is identified in both cases before determining if a terminal disclaimer is appropriate, since this is a provisional rejection.

Additionally, at page 7 of the Office action, the Examiner states, "Patented claims do not recite the concentration of the acid while loading the active agent and instant mM amounts therefore, are deemed to be anticipated by the claims of the patent." It is respectfully submitted that the Examiner's statement is contrary to well established law regarding anticipation.

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Dillon*, 919 F.2d 688, 16 U.S.P.Q.2d 1897, 1908 (Fed. Cir. 1990) (en banc), cert. denied, 500 U.S. 904 (1991). For anticipation, there must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the art. *Scripps Clinic & Res. Found. v. Genentech, Inc.*, 927 F.2d 1565, 18 USPQ2d 101 (Fed. Cir. 1991).

The instant claims are directed to a method of forming gradient loaded liposomes, the method comprising: (a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid.... The claims specifically recite "an aqueous solution of at least about 60 mM of an acid." This element is not found in the claims of U.S. Patent Number 6,740,335. Accordingly, the Examiner's statement regarding the anticipatory effect of the cited claims is incorrect.

CONCLUSION

In light of the above remarks and amendments, withdrawal of the outstanding rejections and allowance of the pending claims 1-28, 30-31, 33, 40-42, and 47-71 is requested. The Examiner is invited to contact Applicant's Representative at the below-listed telephone number if there are any questions regarding this Response or if prosecution of this application may be assisted thereby.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 50-3503. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extension fees to Deposit Account 50-3503.

Respectfully submitted,

Ning Hu et al.

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